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Review

Tau aggregation is driven by a transition from random coil to beta sheet structure

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Abstract

The abnormal aggregation of the microtubule associated protein tau into paired helical filaments (PHFs) is one the hallmarks of Alzheimer's disease. The soluble protein is one of the longest natively unfolded proteins, lacking significant amounts of secondary structure over a sequence of 441 amino acids in the longest isoform. Furthermore, the unfolded character is consistent with some notable features of the protein like stability towards heat and acid treatment. It is still unclear how these characteristics support the physiological function of binding to and stabilization of microtubules. We review here some recent studies on how an unfolded protein such as tau can adopt β-structure, which then leads to the highly ordered morphology of the PHFs. The core sequence for both microtubule binding and PHF formation is the microtubule binding domain containing three or four repeats. This region alone is sufficient for PHF formation and mostly unfolded in the soluble state. A search for sequence motifs within this region crucial for PHF building revealed two hexapeptides in the second and the third repeat. Some of the genetically linked cases of FTDP-17 show missense mutations in or adjacent to these hexapeptide motifs. Proteins containing the P301L and the ΔK280 mutations exhibit accelerated aggregation. The importance of the two hexapeptides stems from their capacity to undergo a conformational change from a random coil to a beta sheet structure. The increase of beta sheet structure is a typical feature of an amyloidogenic protein and is the basis of other characteristics like a decreased sensitivity towards proteolytic degradation and Congo red binding. PHFs aggregated in vitro and in vivo contain β-sheet structure, as judged by circular dichroism (CD) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction.

Keywords: Alzheimer's disease; Tau; Paired helical filament; FTDP-17; Cross beta structure

1. Introduction

There are 22 different diseases known which are caused by misfolded proteins [1–5]. Among them there are 5 (prion disease, Huntington's disease, Parkinson's disease, Alzheimer's disease and frontotemporal dementias) in which the misfolded protein is predominantly expressed in the brain and the disease is determined by neurodegeneration. In the case of Alzheimer's disease, proteinacous aggregates built from two different proteins are involved: the extracellular amyloid plaques consisting of filaments of the Aβ-peptide, and the intracellular neurofibrillary tangles and neuropil threads formed by filaments from the microtubule-associated protein tau (termed paired helical filaments, PHFs).

Besides Alzheimer's disease which requires both types of aggregates for a positive postmortem diagnosis, there is a group of frontotemporal dementias characterized by aggregated tau protein in the brain. In the familial cases, two groups of mutations in the tau gene are found: the intronic ones influencing the ratio of isoforms containing three or four repeats and the exonic ones causing missense mutations or deletions [6,7].

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; CD, circular dichroism; EM, electron microscope; FTIR, Fourier transform infrared; PHF, paired helical filaments; ThS, thioflavin S

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The Aβ peptide is a proteolytic fragment of amyloid precursor protein (APP) spanning a transmembrane domain consisting mainly of hydrophobic amino acids and thereby prone to aggregation. The microtubule associated protein tau, in contrast, is a highly soluble protein largely devoid of hydrophobic amino acids. The percentage of the hydrophobic amino acids leucine, isoleucine, phenylanlanine, tryptophan and valine is only ~15%, compared with about 30% for a typical globular protein. In addition, tau contains an excess of positive charged amino acids resulting in a pI of 8.3-10.0 depending on the isoform. The lack of hydrophobic amino acids and the net charge at physiological pH explains the unfolded character of the soluble tau protein [8,9]. Among the increasing number of natively unfolded proteins or protein domains, tau is one of the largest [10].

The physiological function of binding and stabilization of microtubules requires the interaction of tau with the surface of the well folded protein tubulin. Here the basic sequences of the three or four repeats interact with the acidic C-terminus of tubulin, which can be extensively polyglutamylated. One tau molecule can interact with several tubulin dimers and thus requires an extended conformation, consistent with the low content of hydrophobic amino acids. But under special circumstances this small degree of hydrophobicity is sufficient to drive tau into aggregation. This explains why the polymerization is highly temperature dependent [11] and tryptophan residues inserted into the PHF core undergo a blue-shift upon polymerization [12]. The aggregation of tau is highly accelerated by the addition of polyanions. These can be polysaccharides like heparin [13], peptides like polyglutamate [11], nucleic acids like RNA [14] or fatty acids micelles like arachidonic acid [15]. Their common feature is an extended negative charge. All of them promote the aggregation of tau in a concentration dependent manner. For heparin the stoichiometry of 4:1 (protein/heparin) is most effective. In this stoichiometry a heparin molecule (average mol. weight of 3000 Da) carries 27 negative charges and is thereby capable to neutralize about 7 positive charges from one tau sequence in a 4:1 complex. Interestingly, a mutation linked with familial FTDP (ΔK280) leads to a deletion of a lysine and this loss of a single positive charge makes this protein capable to polymerize readily without the need of a polyanion [16]. In the case of arachidonic acid, the presence of tau lowers the critical micelle concentration (CMC) and the micelles are the active species for promoting tau aggregation [17]. Similarly, beads with a negatively charged surface also stimulate tau aggregation [18].

The interaction with polyanions leads to a conformational switch from mostly random coil to a beta sheet structure in some parts of the sequence. We identified two hexapeptide motifs in the second and the third repeat of tau, which are crucial for this change in conformation [19]. The beta sheet conformation in the polymers is an essential feature defining amyloid. In PHFs made from full-length tau, the beta structure is difficult to detect by X-ray or spectroscopic methods [20,21]. The reason is that only a small portion of the sequence adopts a beta structure, while the rest of the sequence stays in a mostly random coil conformation after PHF assembly. With the restriction of the sequence to the minimal PHF building block, the fraction of β-structure increases and becomes clearly visible [16,19,22,23]. We believe that these results, combined with those of others [24–27], settle a long-standing question and show that PHFs can be considered as "amyloid" and, in this regard, are similar to most other proteins that aggregate in a pathological fashion (review Refs. [28,29]).

2. Materials and methods

2.1. Chemicals and proteins

Heparin (average MW of 6000), polyglutamate (average MW of 600 or 1000), and thioflavin-S were obtained from Sigma. Full-length tau isoforms htau23, htau24 and constructs of the repeat domain of tau (Fig. 1) were expressed in *E. coli* and purified by making use of the heat stability and FPLC Mono S (Pharmacia) chromatography as described [30]. The purity of the proteins was analyzed by SDS-PAGE; protein concentrations were determined by the Bradford assay and by analysis of absorbance at 214 nm.

2.2. PHF formation in vitro

Assembly of synthetic PHFs from tau protein (K19, 10 μM) was performed at 37 °C in the presence of

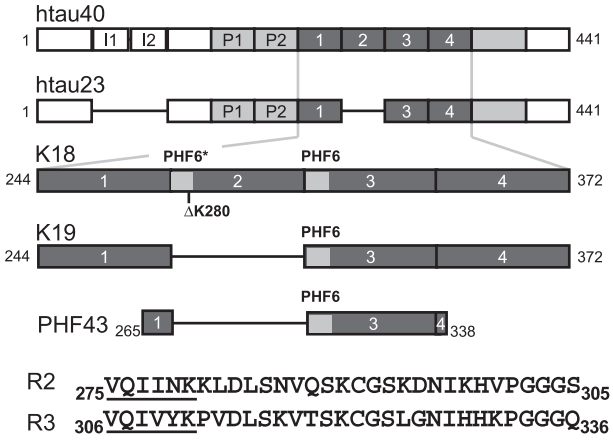

Fig. 1. Constructs and isoforms of tau. The isoform htau40 is the longest isoform in the human CNS (441 residues), containing two N-terminal inserts and four repeats of 31 or 32 residues in the C-terminal half (Goedert et al. [36]); htau23 is the shortest isoform. Construct K18 comprises the sequence of four repeats; construct K19 contains three repeats. The peptide PHF43 can be obtained by proteolysis of K19 with the endoprotease GluC. The sequence of the second and the third repeat is shown at the bottom, the two hexapeptides PHF6 (V305-K311) and PHF6* (V275-K281) are underlined. Note that R2 corresponds to the alternatively spliced exon 10.

polyanions (heparin; 5 μM) in 50 mM NH_4Ac, pH 6.8. Assembly was followed either qualitatively by electron microscopy or quantitatively by fluorescence assay using thioflavin-S. PHF-formation from tau isoforms htau23 and htau24 was carried out in PBS-buffer pH 7.4, 50 μM protein, and 12.5 μM heparin. The samples were incubated at 50 °C for 10 days. In the case of hTau24 and K18, DTT was added at a final concentration of 1 mM each day in order to avoid intramolecular disulfide cross-linking [31].

2.3. Alzheimer PHFs

Immunoaffinity-purified PHFs were a gift from Dr. P. Davies (Albert-Einstein College of Medicine). They were prepared from three Alzheimer's disease brains as described, using the reaction with the MC-1 antibody [32,33]. The Alzheimer PHFs were concentrated by pelleting at 86 000×*g* for 14 h and resuspended in PBS. The purity of the PHF preparation was analyzed by SDS-PAGE and Western blotting with the rabbit polyclonal pan-tau antibody K9JA (Dako Diagnostics, Hamburg, Germany) raised against the four repeats and the C-terminal tail of tau.

2.4. Electron microscopy

For ascertaining PHF assembly in vitro and to examine the overall appearance of Alzheimer PHFs, 10 μl protein solutions diluted to 1–10 μM protein were placed on 600-mesh carbon-coated copper grids for 45 s, washed twice with H_2O and negatively stained with 2% uranyl acetate for 45 s. The specimens were examined with a Philips CM12 electron microscope at 100 kV.

2.5. Fourier transform infrared (FTIR) spectroscopy

FTIR experiments were performed on a Jasco FTIR-410 instrument (Jasco, Groß-Umstadt, Germany). Atmospheric water vapor was removed by flushing the spectrometer with nitrogen. Interferograms were recorded between 1700 and 1600 cm^{-1}, and 128 spectra were averaged. They were acquired in the transmission mode using CaF2 cells separated by spacers of 25 μm. After recording a reference spectrum of the instrument and of the fresh D_2O lot used, the protein solutions were applied, and the absorbance spectrum of the sample was measured. The D_2O spectra and sample spectra were first corrected for the water vapor background before sub-

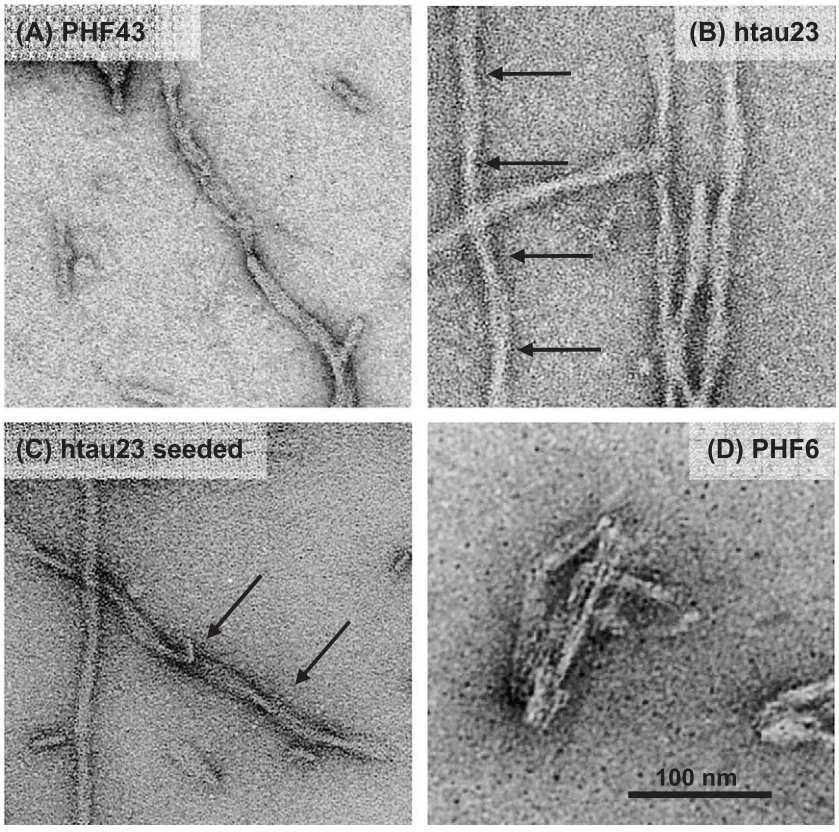

Fig. 2. Electron micrographs of tau aggregation products. (A) PHF43 (comprising N265-E338 but lacking R2) and htau23 (B). The filaments from PHF43 lack the typical twisted appearance of paired helical filaments which are visible in the htau23 sample. However, seeds prepared from PHF43-filaments can seed htau23 into bona fide paired helical filaments (C). The synthetic peptide comprising the sequence of the hexapeptide PHF6 forms amorphous aggregates under polymerization conditions (D). Bar=100 nm.

tracting the D_2O spectra from the sample spectrum. To facilitate comparison, the spectra were then normalized with respect to their maxima. Typical concentrations (measured by absorption at 214 nm) were 3–6 mg/ml (70–600 μM, depending on tau-construct and tau-isoform) for soluble tau protein and for reassembled or brain-derived PHFs.

2.6. Circular dichroism (CD) spectroscopy

All measurements were carried out with a Jasco J-715 CD-spectrometer (Jasco, Groβ-Umstadt, Germany) in a cuvette with 0.05-cm path length. The scanning speed was 100 nm/min with a band width of 1.0 nm and a response time of 0.5 s. In each experiment 25 spectra were summed and averaged. To minimize signal to noise ratio in the lower UV range, a second spectrum from each sample was recorded between 194 and 200 nm with a scanning speed of 20 nm/min at a band width of 1.0 nm and a response time of 1.0 s. In this case 100 spectra were summed and averaged. The two final spectra for each sample were then joined together using the CD-software implemented "concatenate" function. For calculation of the mean residue ellipticity, the protein concentration was obtained by using the second channel of the CD-spectrometer to measure the absorption of the protein sample at 214 nm (where absorption is dominated by the peptide bonds). Calibration of A_{214nm} was done by BSA standards. The secondary structure interpretation of the CD data was performed with the program Dicroprot (http://www.dicroprot-pbil.ibcp.fr) [34] which allows fitting against standard CD spectra [35] as well as comparison with a set of known proteins.

2.7. X-ray diffraction

K18-ΔK280 was allowed to polymerize into PHFs using concentrations of at least 500 μM in the absence of heparin, centrifuged at 100,000×g. The pellets were placed into the wide end of a borosilicate capillary tube with a diameter of 0.5 mm and pushed down using a 2% agarose cushion. The samples were dried at room temperature while placed in the field of a 2T permanent magnet (Hummingbird Instruments, Arlington, MA, USA). Diffraction patterns were recorded on a rotating anode X-ray generator (Rigaku U200) operated at 50 kV, 100 mA, λ=1.5418 Å (Cu Kα), and equipped with an image plate detector (MarResearch, Hamburg, Germany).

3. Results

In Alzheimer's disease PHFs contain all six isoforms of tau found in the human CNS [36], as well as some truncated forms [37]. The longest isoform htau40 contains two N-terminal inserts and four repeats in the C-terminal half of the protein (Fig. 1). The shortest isoform htau23 lacks the N-terminal inserts and comprises only three repeats (R1, R3, R4). A construct derived from this isoform with three repeats is capable of forming PHFs in vitro [21,38]. We asked which amino acids within this construct are essential for polymerization. By proteolytic digestion with different proteases, we generated various peptides, purified them by

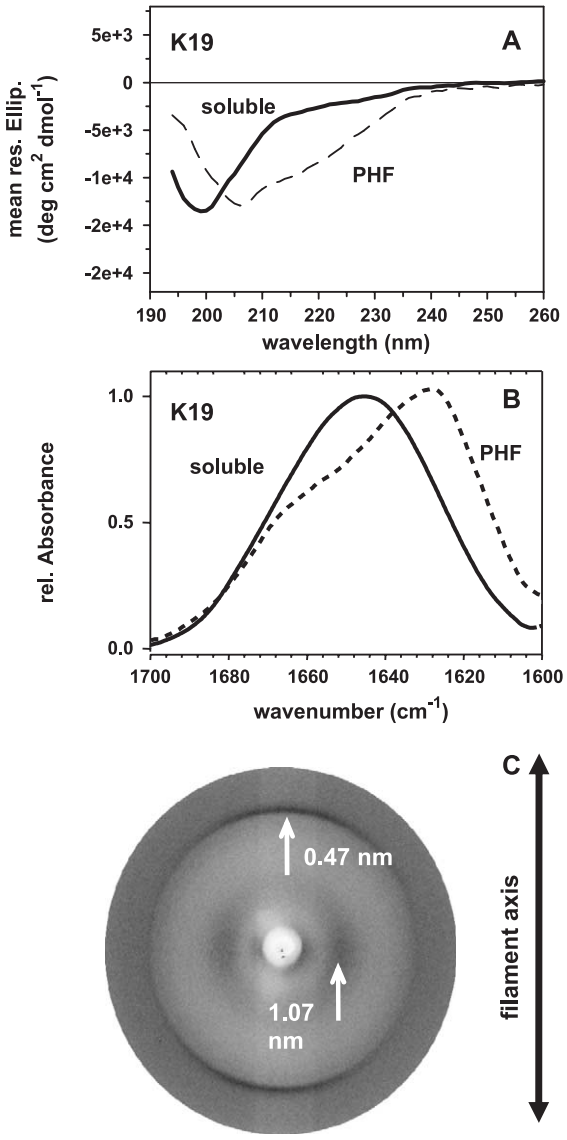

Fig. 3. CD, FTIR, and X-ray diffraction reveal beta structure. (A) The analysis of soluble (straight line) and polymerized (dashed line) K19 by CD shows a clear minimum for the spectrum of the soluble sample at 200 nm, indicating a mostly random coil structure, whereas the minimum is shifted towards higher wavelengths after polymerization, indicating an increase of β-structure. (B) FTIR spectroscopy of soluble (straight line) and polymerized (dotted line) K19 confirms the increase of β-structure upon polymerization, due to the increase of absorbance at about 1630 cm^{-1} after polymerization. (C) X-ray diffraction of PHFs made by K18-ΔK280 reveals the typical cross-beta diffraction pattern with a meridional reflection at 0.47 nm for the distance between two strands within a β-sheet and a reflection at 1.07 nm on the equator indicating the average distance of the intersheet stacking.

reverse phase HPLC and tested them for the capacity to form PHFs [19]. We found that a hexapeptide motif (V305-K311) at the beginning of the third repeat is crucial for PHF assembly. All peptides which show aggregation contained this sequence and a synthetic peptide of this sequence also aggregates rapidly. A similar hexapeptide is present at the beginning of the second repeat R2 (Fig. 1). The minimal sequence to obtain aggregation is a peptide consisting of 43 amino acids called PHF43 (N265-E338, lacking R2=V275-S304), which contains the whole third repeat and the adjacent regions of the first and the fourth repeats.

The polymerization products of PHF43 as judged by electron microscope (EM) are not well-formed PHFs, but filamentous structures with widths of about 8 nm (Fig. 2a). For comparison, the polymerization product of htau23 exhibits a regular twist every 80 nm (Fig. 2b). Although PHF43 is not sufficient to display the typical morphology, it is capable to seed polymerization of htau23. The PHFs formed by seeded polymerization show the twisted appearance of bona fide PHFs (Fig. 2c). The peptide PHF6 (V305-K311) forms amorphous aggregates and is not capable to seed polymerization of longer constructs. The same peptide sequence with an acetylation at the N-terminus and an amidation at the C-terminus is capable to form filamentous structures [25]. The peptide PHF43 contains the structural information which is necessary to start polymerization but lacks the determinants to form bona fide PHFs. Therefore, we asked what kind of structural information is needed for PHF assembly.

Using the aggregated PHF43 peptide, we found a gross change of secondary structure by CD spectroscopy [19]. This change was missed in earlier experiments [21] because at that time only larger constructs and full-length PHFs were studied where the signal from the local beta structure was buried by the surrounding random-coil structure. In the case of the three-repeat construct K19, it is necessary to separate the PHFs from the soluble protein in the reaction by centrifugation prior to CD spectroscopy in order to detect the difference in the spectra (Fig. 3a). The soluble protein exhibits a minimum around 200 nm indicating a mostly random coil structure. The spectrum of the PHF sample exhibits a clear shift of the minimum to higher wavelengths. Such a broad minimum at ~215 nm is a typical feature of beta sheet structure. The result was confirmed by the analysis of the soluble and polymerized protein by FTIR-spectroscopy (Fig. 3b). The soluble protein exhibits a maximum at 1650 cm^{-1}, consistent with random coil structure, whereas the PHF sample peaks at 1630 cm^{-1}, also clearly indicating an increase of beta structure.

The organization of the beta structure within the polymer was analyzed by X-ray diffraction. The PHFs formed by the construct K18-ΔK280 were partially aligned by drying in a magnetic field. This results in pronounced reflections centered at 0.47 nm on the meridian and 1.07 nm on the equator (distributed over arcs due to the imperfect orientation of the fibers). The reflection at 0.47 nm represents the distance between two adjacent strands in a beta sheet; the 1.07-nm reflection typically stems from the intersheet stacking of two beta sheets [39].

The next step was to analyze PHFs aggregated in vivo and purified from Alzheimer brain tissue. Here the most difficult problem is the purification of the samples because every impurity (protein or other components) can have a strong impact on the CD and FTIR analysis. This question is of special interest because the secondary structure of tau within the PHFs was a matter of debate. Other authors have used a multiple step procedure including centrifugation, solubility in detergents and density gradient centrifugation [40]. For our analysis we used PHFs obtained by a one-step immunoaffinity based

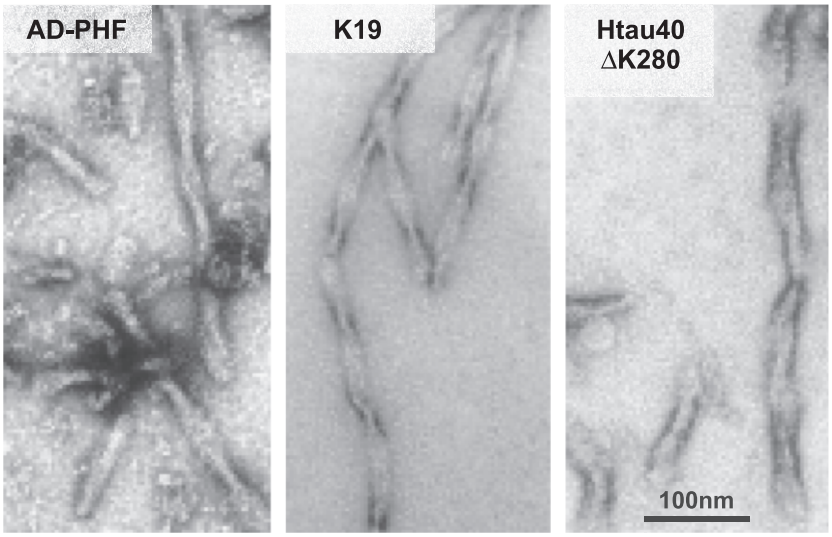

Fig. 4. Negative stain EM of PHFs assembled in vivo and in vitro. Left, PHFs purified from Alzheimer brain tissue. Middle, filaments derived from construct K19. Right, PHFs assembled from htau40. Most fibers show the typical paired helical appearance of brain-derived Alzheimer PHFs, with a width of 10–25 nm and a crossover repeat of about 80 nm. Bar=100 nm.

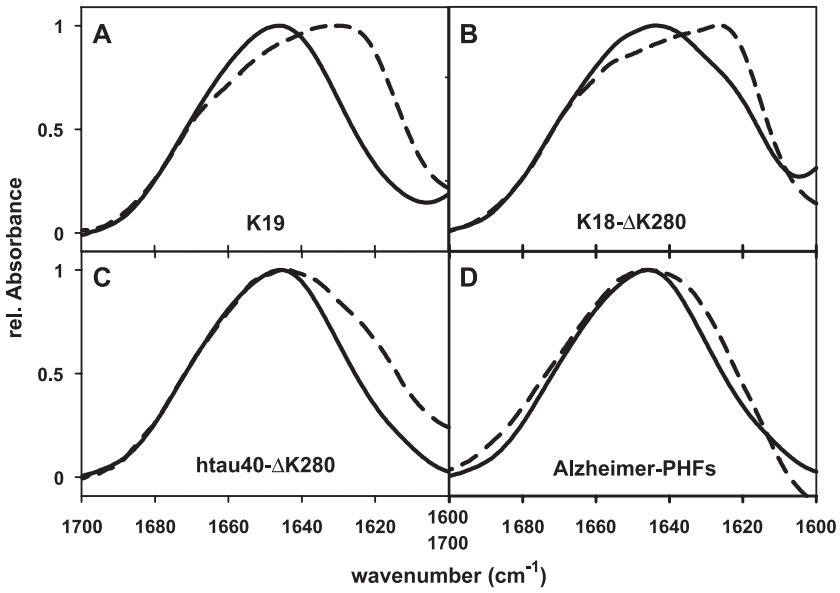

Fig. 5. FTIR spectra of PHFs from Alzheimer brain tissue, reassembled from recombinant tau, and soluble tau. (A) Three-repeat construct K19, (B) four-repeat construct K18-ΔK280, (C) full-length isoform htau40-ΔK280, (D) AD-PHFs. Soluble tau (solid curves) shows spectra with maxima at ~1645 cm^{-1} indicating a random coil structure of the protein. By contrast, the corresponding spectra of PHFs reassembled in vitro are shifted to the right (lower wave numbers) with maxima or shoulders around 1630 cm^{-1} (dashed lines in A–C) indicating an increase in β-structure during PHF-assembly. For measuring the FTIR-spectrum of the Alzheimer PHFs (D), three individual cases were pooled to increase the PHF concentration to ~3 mg/ml. The spectrum shows an additional shoulder around 1630 cm^{-1} indicating increased β-structure.

procedure [41], which has been proven by SDS-PAGE and Western blot analysis to be highly pure [22]. By electron microscopy these filaments exhibit the typical twisted appearance (Fig. 4a). Compared with PHFs assembled in vitro derived from K19 (Fig. 4b) or from htau40-ΔK280 (Fig. 4c), the PHFs from Alzheimer's brain tend to be shorter, possibly due to breakage or as a result of disassembly and reassembly during purification.

The spectra obtained by FTIR-spectroscopy show a maximum around 1650 cm^{-1} for the soluble protein and a shoulder at 1630 cm^{-1}, present only in the aggregated samples, which depends on the length of the protein (Fig. 5). For K19 consisting of the three repeats the PHF sample shows a shift of the maximum to 1625–1630 cm^{-1}, whereas for the full-length htau40DK280 the relative absorbance at these wave numbers results in a shoulder of the peak which is still centered around 1650 cm^{-1}. We have shown that the repeats are sufficient to form beta structure and these results show that the fraction of the repeats compared with the whole sequence determines the fraction of beta sheet observed in the spectra. The rest of the protein seems to stay in a mostly random coil structure. The spectrum representing the in vivo PHFs also exhibits a shoulder at about 1630 cm^{-1}, compared to the soluble full-length tau protein, indicating an increase of beta sheet structure.

4. Discussion

The discovery of the role of the hexapeptides and their conformational switch to beta structure explains the amyloidogenic features of aggregated tau, the enhanced resistance against proteolytic digestion and the binding of thioflavin-S. In this regard, tau turns out to be similar to other amyloidogenic proteins where short core sequences are considered to be crucial for the pathological aggregation [29,42–44]. The importance of the hexapeptides for the beta conformation has in the meantime been confirmed by the detailed analysis of synthetic peptides of various lengths [45]. The formation of filaments by shorter peptides enabled the authors to analyze partly orientated filaments by X-ray diffraction in order to determine the molecular structure. It was found that the distances between the beta strands showed nearly no variation but the intersheet stacking varied from 0.81 to 0.97 nm. By obtaining an improved orientation of the filaments, the arrangement within the PHF core can be solved.

A model for the arrangement of short beta sheet elements and the other parts of the sequence was recently presented based on EPR spectroscopy of spin-labeled tau aggregates [26]. Their model is built on the structure of carbonic anhydrase which incorporates a triple beta helix. In this, the beta sheet elements are arranged around a triangular cross section and are connected by protruding loops of unknown structure. A similar model was suggested for the organization of aggregated prion protein [46]. The results would be consistent with the observation that the aggregation of tau can be by poisoned by insertion of prolines into the hexapeptide motifs which break beta structure [16]; however, if only one motif is poisoned in a four-repeat construct the other is still capable to drive PHF formation [12] (Fig. 6).

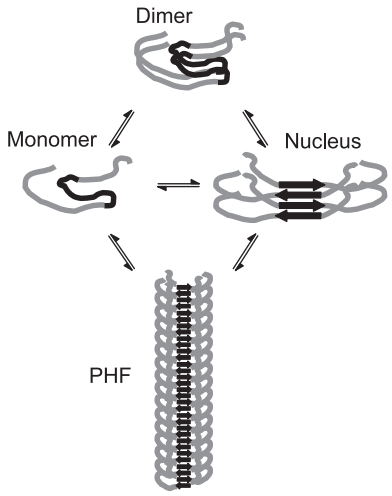

Fig. 6. Model of PHF formation. Monomers of tau are unfolded (repeats shown in black) and can dimerize without a substantial change in secondary structure. When the nucleus of aggregation is formed, an increase of β-sheet takes place (black arrows) and the nucleus can be elongated to build PHFs. PHF assembly is in principle reversible, disassembly could take place by release of tau monomers, dimers, or oligomers.

It is currently unclear what fraction of the tau sequence is engaged in forming beta strands. Are they limited only to the two hexapeptide motifs or can other parts of the sequence adapt to beta structure as well? The CD and FTIR data suggest that there is about 30% of beta structure in the 120-residue sequence of the repeat domain. Although quantification of secondary structure elements by CD and FTIR is not very accurate, these percentages cannot be reached by the hexapeptides alone. We therefore assume that other regions of the protein can also be converted into beta structure. For example, it has been suggested that the end of the third repeat contributes to the core structure of the PHFs [47].

Although we found the structure of the soluble PHF43 dominated by random coil, there might be an intrinsic tendency to form beta structure. NMR spectroscopy showed that the third repeat, and especially the hexapeptide sequence, is resistant to the formation of alpha helical structure induced by addition of TFE [48]. This means that there might be some residual structure already in the soluble state, which is not detectable by averaging techniques like CD-spectroscopy or FTIR.

Which events trigger the transition from random coil to beta sheet? First, the transition is concentration-dependent. The hexapeptide is able to interact with itself [19], which explains the concentration dependence. Second, the transition can also be regulated by an intermolecular or intramolecular interaction. The physiological interaction partner of the repeats is the C-terminal domain of tubulin which is exposed on the microtubule surface. In the presence of microtubules, tau can accumulate on the surface and adopts a PHF-like conformation (as judged by thioflavin-S staining), but does not form filaments [49]. Even when bound to microtubules, tau remains mostly in the random coil conformation and covers up a large fraction of the surface [50].

One intramolecular interaction partner of the repeat domain is the N-terminal domain of tau, as shown by conformation-dependent antibodies such as Alz50, suggesting that pathological tau can adopt a folded conformation. This antibody is used for the diagnosis of early changes in Alzheimer brain tissue [51]. The antibody binds to a nonlinear epitope consisting of near-N-terminal residues and the repeat domain [52]. Another conformation-dependent antibody is MC1, which recognizes tau in a pathological conformation and serves as a marker of early stages in the development of Alzheimer's disease [53]. Another possible interaction can occur with a proposed amphipathic alpha helix near the C-terminus [54]. The presence of the C-terminal region slows down PHF assembly and this effect can also be achieved by an excess of peptides mimicking the C-terminal region [55]. As a consequence, cleaving the C-terminus by a caspase can stimulate tau polymerization [56]. This is reminiscent of the case of α-synuclein where the C-terminus binds to the polymerizing core fragment in the middle of α-synuclein, and the polymerization can be accelerated by polycations that interfere with this interaction [57].

Tau purified from Alzheimer's disease brain is hyperphosphorylated [58] and it is still unclear whether the phosphorylation is cause or effect of PHF formation. Tau protein contains about 21 different reported phosphorylation sites [59], which can be classified into proline- and non-proline-directed sites. The non-proline-directed sites include phosphorylation sites on sequence motifs KXGS within the repeats which are known to regulate the affinity to the microtubules [60]. Phosphorylation in the repeats strongly inhibits tau-polymerization; phosphorylation at other sites is also inhibitory but less efficient [61]. However, the issue continues to be a matter of debate since other groups reported a stimulating effect of a mixture of different phosphorylation sites [62]. The phosphorylation is achieved by different kinases so that the phosphorylation represents a mixture of sites whose relative weight is difficult to quantify. A reliable method to analyze the influence of phosphorylation onto the structure of tau is the simulation of phosphorylated residues by glutamate mutations [63]. The mutations examined in this study did not lead to a gross change in conformation but were able to decrease the binding capacity towards microtubules.

The content of beta sheet structure within PHFs was recently questioned by results pointing to a high content of α-helical structure [40]. In this study, PHFs were analyzed by CD spectroscopy and FTIR-spectroscopy, from which mainly the CD-spectra supported the alpha helix structure as a main component of the PHFs. Our results were obtained from PHFs purified by affinity-chromatography and confirmed by another study which used material from the same source [25]. These data argue against a substantial content of α-helical structure in PHFs in vitro and in vivo.

Overall fraction of secondary structure is small in PHFs assembled in vitro by full-length tau protein as well as in PHFs from Alzheimer brain tissue. However, the local increase of beta structure in PHFs places tau on the list of amyloidogenic proteins. This knowledge of the principles of tau polymerization will help to address open questions on the molecular structure of the PHFs, the impact of phosphorylation and the methods to inhibit the pathological aggregation of tau.

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